

This Page Is Inserted by IFW Operations
and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

**As rescanning documents *will not* correct images,
please do not report the images to the
Image Problem Mailbox.**

1 540 376

- (21) Application No. 6979/75 (22) Filed 19 Feb. 1975
 (23) Complete Specification filed 18 Feb. 1976
 (44) Complete Specification published 14 Feb. 1979
 (51) INT. CL.³ C07G 7/00
 (52) Index at acceptance
 C3H G

(72) Inventors PETER HARRIS
 PETER JOHN LILLFORD
 ROBERT MIDDLETON LIVINGSTON
 DAVID JOHN WRIGHT



(54) CONCENTRATION OF PROTEIN SOLUTIONS BY
 ULTRAFILTRATION

(71) We, UNILEVER LIMITED, a company organised under the laws of Great Britain, of Unilever House, Blackfriars, London E/C 4, England, do hereby declare the invention for which we pray that a patent may be granted to us and the method by which it is to be performed, to be particularly described in and by the following statement:—

10 This invention relates to the concentration of aqueous solutions of protein.

During the extraction of proteins from raw material, e.g. of a seed protein from defatted oil seed meal, there is usually obtained an aqueous solution of the desired protein which is of fairly low concentration (not more than 5% protein by weight) and contains various dissolved substances, particularly salts, derived from the raw material. Such a solution can be concentrated with respect to protein, and simultaneously purified by partial or substantially complete removal of the dissolved salts, by the procedure known as ultrafiltration (sometimes called membrane ultrafiltration). In essence, the protein solution is pressurised against a membrane so constructed as to retain the dissolved, large molecules of protein but allow passage of dissolved molecules of low molecular weight—not just those of the solvent (water) but also those of the salts, such as sodium or potassium chloride. For a summary of principles and practice, see chapter 5 entitled "Purification of Proteins by Membrane Ultrafiltration", contributed by Gary J. Fallick to the book "Ultrapurity Methods and Techniques" Ed. M. Zief and R. Speights, Marcel Dekker Inc., New York, 1972. However, in ultrafiltration the rate of concentration decreases with increasing concentration of the retained species (protein), and at a concentration of 10% by weight the rate is very low.

45 We have now found that the concentra-

tion rate can be accelerated at 10% and higher dissolved protein concentration. The present invention therefore provides a process for concentrating a solution of globular protein by ultrafiltration, in which the ultrafiltration is carried out in the presence of an alkali metal salt at an ionic strength of at least 0.25, and preferably at least 0.33. An ionic strength in the range 0.4-0.75 is particularly preferred. Because the product of ultrafiltration (the retentate) will ordinarily find use in the production of foodstuffs, the salt is preferably a non-toxic one, i.e. ingestible without harm. The use of sodium chloride or potassium chloride is particularly preferred. The salt probably exerts its beneficial effect by reducing the viscosity of the protein solution.

The salt can be added to the protein at any convenient stage during processing, for example to the liquor used for extraction of the protein from its source material, but it is best incorporated (at the stipulated minimum ionic strength of 0.25) in the protein solution at ultrafiltration, shortly before a significant decrease in concentration rate would ordinarily be expected to occur. Of course, the concentrate obtained (of 15, 20, 25% or even more by weight of dissolved protein) will have a substantial content of the dissolved salt that has been incorporated, but such salt-containing systems can readily be made use of in the manufacture of food products. Thus, if (as will ordinarily be the case) they have a pH in the range 6-9, particularly 6-8, their pH can be reduced to the range 4-6, by careful addition of acid so as not to produce an unduly high local concentration of acid, to yield heat-setting salt-containing protein solutions of the kind disclosed in our British Patent Specification 1,265,661. Such solutions are heat-settable not only in bulk, but also on extrusion into hot water to yield fibre.

90

The invention is applicable to the concentration by ultrafiltration of a wide range of globular proteins (globulins), particularly the proteins of oil-bearing seeds such as soy, groundnut, rape, sunflower, cottonseed, safflower, and field beans.

The invention is further illustrated by the following Example.

EXAMPLE

10. One part by weight of a low-temperature solvent-extracted soybean meal was mixed with 10 parts by weight of water containing a few drops of antifoam agent. The resulting slurry was centrifuged 15 at 5000 g for 20 minutes to remove insoluble carbohydrate material. The supernatant solution (pH 6.5; protein concentration 4% by weight; viscosity 5 centipoise at 20°C) was concentrated using a conventional tubular module ultrafiltration plant 20 working at a temperature of 20°C. [The membrane used was a conventional one of thickness 0.25 mm and median pore size 50μ (50×10^{-4} cm) cast conventionally from a dimethylsulphoxide solution of commercially available cellulose acetate (Eastman Kodak 3983, of acetyl content 39.8% and molecular weight 20,000-30,000). The operation pressure was 60 psig (inlet) and 30 40 psig (outlet).]

When the solution had been reduced to about half its initial volume (i.e. protein concentration was about 8% by weight), sodium chloride was added to a concentration of 3% by weight on the water present 35 i.e. to an ionic strength of 0.5 on the water present. Ultrafiltration was continued until the retentate had a protein concentration of about 18% by weight. The retentate 40 (pH 6.6, viscosity about 50 centipoise at 20°C was brought to pH 4.8 by careful

addition of hydrochloric acid. No precipitation of protein resulted, and the solution remained transparent.

The product of pH 4.8 was introduced 45 dropwise into a stream of hot water (temperature about 98°C), and long filaments of heat-set protein were produced, suitable for incorporation in meat-simulating products as described for example in our 50 British Patent Specification 1,265,661).

WHAT WE CLAIM IS:

1. A process for concentrating a solution of globular protein by ultrafiltration, in which the ultrafiltration is carried out 55 in the presence of an alkali metal salt at an ionic strength of at least 0.25.
2. A process according to Claim 1, in which an alkali metal chloride is used.
3. A process according to Claim 2, in which sodium chloride is used.
4. A process according to Claim 1, 2 or 3, in which the ionic strength is at least 0.33.
5. A process according to any one of the 65 preceding claims in which the ionic strength is in the range of 0.4-0.75.
6. A process according to any one of the preceding claims in which the protein solution contains at least 10 wt % dissolved 70 protein.
7. A process according to any one of the preceding claims in which the protein comprises soy protein.
8. A process as claimed in Claim 1 substantially as described with particular 75 reference to the Example.
9. Globular protein whenever concentrated by a process as claimed in any of the preceding claims. 80

D. LITHERLAND,
Chartered Patent Agent.